A Novel Plant Extract Mix is Capable of Binding Endotoxin

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BACKGROUND: Lipopolysaccharide (LPS), the major component of endotoxin, is present in the outer membrane of Gram-negative bacteria and can trigger immune response by interacting with LPS receptors on the surface of immune cells. Too much endotoxin release in the presence of an overwhelming Gram-negative bacterial infection can contribute to life-threatening inflammatory reactions, diarrhea, endotoxemia, and/or shock. Tannin compounds have been found to interfere with bacterial adhesion by blocking LPS receptors.

A novel plant extract, Grazix feed supplement (LiveLeaf, USA), is believed to be capable of binding LPS by inhibiting the binding between LPS and its target receptors. Consumption of Grazix has reduced incidence of scour in weaned piglets but its mechanism has not been determined.

OBJECTIVE: To quantify the LPS-binding potential of the novel plant extract mix Grazix feed supplement.

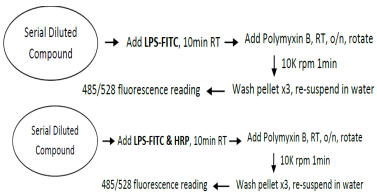


Figure 1. Serially diluted compounds were mixed with LPS-FITC and polymixin B (with or without HRP) to determine inhibition in the binding between LPS-FITC and polymixin B.

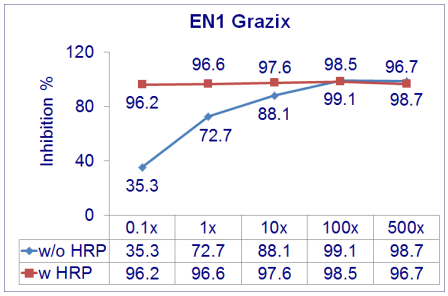
METHODS: A LPS binding inhibition assay was developed specifically for this study. The binding of fluoresein isothicyanate conjugated LPS (LPS-FITC) to a potent LPS-binding molecule, polymixin B, immobilized by agarose beads is shown to be inhibited by the compound tested if the precipitate of a complex with LPS-FITC and polymixin B-agarose beads is reduced. By comparing the fluorescent signals, the inhibition capability of the tested compound on the binding between LPS-FITC and polymixin B agarose beads can be determined.

A simple of Grazix feed supplment was diluted in sterile distilled water 1000x, 100x, 10x, 1x, and 0.1x and then mixed with LPS-FITC, with or without horseradish peroxidase (HRP), and incubated at room temperature (RT) for 10 minutes. As a control, a tannin compound was mixed in a similar fashion (Figure 1).

Polymixin B was added to the mixtures and incubated at RT overnight. After centrifugation, the supernatants were discarded and pellets containing polymixin B bound by LPS-FITC were resuspended in distilled water.

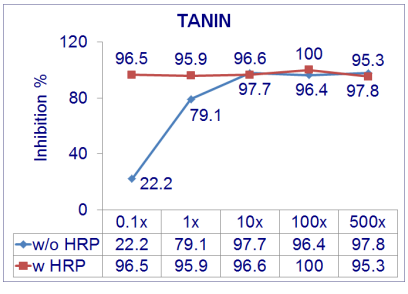
The fluorescence of the suspension mixture was measured in a microplate reader. The percentage of polymixin B/ LPS-FITC binding inhibition was calculated by subtracting the percentage of polymixin B/LPS-FITC binding detected from 100%.

RESULTS: The binding potential of Grazix and the tanin compound increased as their concentrations increased without the addition of HRP (Figures 2 & 3); addition of HRP enhanced the LPS binding activity of both compounds.



	0.1x	1x	10x	100x	500x
w/o HRP	35.3	72.7	88.1	99.1	98.7
w HRP	96.2	96.6	97.6	98.5	96.7

Figure 2. Inhibition of binding between LPS-FITC and polymixin B-agarose beads by Grazix in serial diluted concentrations with and without the presence of HRP.



	0.1x	1x	10x	100x	500x
w/o HRP	22.2	79.1	97.7	96.4	97.8
w HRP	96.5	95.9	96.6	100	95.3

Figure 3. Inhibition of binding between LPS-FITC and polymixin B-agarose beads by Tannin in serial diluted concentrations with and without the presence of HRP.

CONCLUSION: The novel plant extract, Grazix feed supplement, displayed a level of LPS binding inhibition comparable to that of tannin, which is known to interfere with bacterial adhesion. These results demonstrate that the novel plant extract, Grazix feed supplement is capable of binding endotoxin.